BACTERIOLOGICAL REVIEWS
Vol. 28, No. 4, p. 444–451 December, 1964

EMERGING PERSPECTIVE OF RUBELLA[1]

FRANKLIN A. NEVA, CHARLES A. ALFORD, JR., AND THOMAS H. WELLER

Department of Tropical Public Health, Harvard School of Public Health, Boston, Massachusetts

INTRODUCTION

"There is probably no class of diseases in which inaccurate diagnosis is more frequently made than in those of the skin; nor is error more likely—than in that subdivision of cutaneous complaints, which have been denominated Exanthemata, or Rashes" (18).

The above quotation, from Maton's paper describing rubella as a clinical entity and delivered before the Royal College of Physicians in 1814, anticipated remarkably some of the problems of the subsequent century and a half with respect to clinical differentiation of exanthem diseases. For many years, diagnostic difficulties with respect to the various exanthemata served as little more than embarrassment or intellectual discomfort to the physician. After Gregg's discovery of the relationship between maternal rubella and congenital defects (6), however, the practical implications of rubella stimulated an increasing effort towards specific identification of the viral exanthemata. Undoubtedly, the sophistication of recent epidemiological studies has contributed to a more realistic estimate of the risks of maternal rubella. However, such information has raised as many questions as it has answered. As with every infectious disease, the availability of specific diagnostic tests, an understanding of the mechanism of pathogenesis, and the development of prophylactic measures all depend upon specific characterization of the etiological agent.

Although there has long been experimental evidence indicating a viral etiology of rubella (8, 7, 2, 11), characterization of the causative agent was accomplished only recently. Since the dual report of isolation of rubella virus in 1962 by Parkman, Buescher, and Artenstein (24), and independently by Weller and Neva (36), confirmatory findings have quickly followed (35, 30, 33, 19). Even though these data are rapidly being extended by current studies, it may be propitious, at this time, to review some of the recently available information on rubella. From the groundwork of clinical, laboratory, and epidemiological investigations, now based upon specific laboratory methods, will the true perspective of rubella finally emerge.

ISOLATION AND PROPAGATION OF THE VIRUS

The methods in current use for isolation and propagation of rubella virus derive from the two basically different techniques originally employed for detecting the presence of the virus. One was based upon direct recognition of cytopathic effects (CPE) produced in cultures of primary human amnion (PHA) cells (36); the other procedure, also called the indirect or exclusion technique, utilized the finding that rubella-infected cultures of grivet monkey kidney cells (GMK) were resistant to challenge with ECHO-11 virus (24). Subsequently, modifications in both techniques have been introduced by other workers. McCarthy et al. found a special line of rabbit kidney cells (RK_{13}) to exhibit rubella-induced CPE (19). The interference procedure has been found applicable to kidney-cell cultures from other species of monkeys and cell lines thereof (35, 30), and other enteroviruses, such as Coxsackie A-9, can be used as challenge virus (30, 33, 19).

[1] A contribution to the Symposium on "Current Progress in Virus Diseases" presented as part of the program for the Centennial of the Boston City Hospital, 1 June 1964, with Maxwell Finland serving as Consultant Editor, and John H. Dingle and Herbert R. Morgan as moderators.

Up to the present time, one or another variation of the interference method has been most widely used for isolation of rubella virus. Despite nonspecificity of the interference procedure, reports have not indicated this to be a serious problem in practice, but more extensive experience is needed. The relatively short period of 10 to 14 days before tentative results are obtained constitutes a distinct advantage of the exclusion technique. It is, of course, mandatory that the identity of all presumptive rubella isolates obtained by the interference procedure be confirmed by acceptable serological tests.

The use of PHA cultures for isolation and growth of rubella virus offers the advantage that presence of virus is indicated directly by the distinctive cytopathology observable in both fresh and stained preparations. Another advantage is that the presence of rubella virus in PHA cultures may also be detected by the interference phenomenon upon addition of Sindbis virus (36). Thus, PHA cultures may be used in a combined direct and indirect system for detection of rubella virus, permitting parallel studies of the phenomena (22). The limitations of this method include the necessity for cell cultures of good quality that contain confluent monolayers of cells, and the requirement that an experienced observer be available for the detection of the involved cells, which are not always abundant in infected cultures. Use of chemically defined, instead of bovine embryonic fluid, medium may result in diminished or suppressed CPE (36).

Reports, now available only in abstract form, covering studies by Giles et al. at Willowbrook State School and by Buescher et al. (4) at the Walter Reed Army Institute of Research indicate that rubella virus is present in the blood or throat of patients as early as 6 or 7 days before appearance of the rash, and disappears from the blood shortly after the exanthem, but may persist in the pharynx as long as 7 days after onset of rash. The virus has also been recovered from the feces and may be present in the urine at the time of the exanthem. However, virus is most easily recovered from the throat, and this source now appears preferable for the attempted isolation of the responsible agent.

No published data are available assessing the comparative effectiveness of different procedures for isolation of rubella virus from clinical specimens. Several methods, including the use of RK_{13} cell line cultures, were successfully employed in one study (19), but not in a comparative fashion. Preliminary results of Burnett and Alford in this laboratory suggest that interference in GMK is more rapid than either the indirect or direct procedures with PHA cultures, and may be more sensitive for the detection of minimal amounts of rubella virus. Critical studies to select sensitive methods for the detection of both wild and in vitro-propagated strains of rubella virus will assume increasing importance in future work related to development of vaccines.

In evaluating the relative merits of different procedures for isolation and propagation of rubella virus, it is important to recall that the indications for the use of a particular virus-host cell system may be variable. Thus, the optimal system for isolation of virus from clinical specimens may not be the best method for the assay of neutralizing antibodies, production of antigen, study of viral kinetics, or demonstration of cytopathology.

CHARACTERISTICS OF RUBELLA VIRUS

On the basis of available centrifugation, filtration, and electron microscopic studies, it would appear that infectious rubella virus particles are relatively large, in the range of 100 to 300 mμ (24, 36, 23, 25), although one report indicates that some infectious particles may be less than 100 mμ in size (19). In the presence of 2% serum, the inactivation rate of rubella at 37 C was found to be 0.3 to 0.4 log ID_{50} per hour (25), but in other media with higher protein content virus infectivity was not completely destroyed after 1 hr at 56 C (36). Some loss in infectivity occurs on storage for several weeks at 4 and -20 C (36), but the virus is stable for many months at -60 C (25). Rubella virus is capable of replication in the presence of 5-iodo-2′-deoxyuridine, and infectious virus is destroyed on exposure to ether, chloroform, and sodium deoxycholate.

The most distinctive cellular alterations attributable to rubella virus are those occurring in infected PHA cultures (36). At any particular time, relatively few of the cells in an infected culture exhibit changes in either fresh or stained preparations. Affected cells appear enlarged or rounded in the fresh culture, and will often exhibit amoeboid pseudopods whose cytoplasmic contents are transparent and may show the presence of round inclusions. When stained, the involved cells show various abnormalities, including disappearance of the nuclear membrane,

prominent clumping of nuclear chromatin, and the presence of round or irregular cytoplasmic, eosinophilic inclusions. The progression of rubella CPE in PHA cultures is slow, but the cytopathic process can be enhanced by continued in vitro passage of the virus. After 15 to 20 serial transfers, we generally find that rubella strains produce 50% or more destruction of the cell sheet within 3 weeks after inoculation (Fig. 1).

Rubella virus can also multiply in cultures of bovine embryonic tissues (22), as well as in those derived from various human and simian sources, and the special cell line of rabbit kidney already mentioned. With most systems, maximal yields of

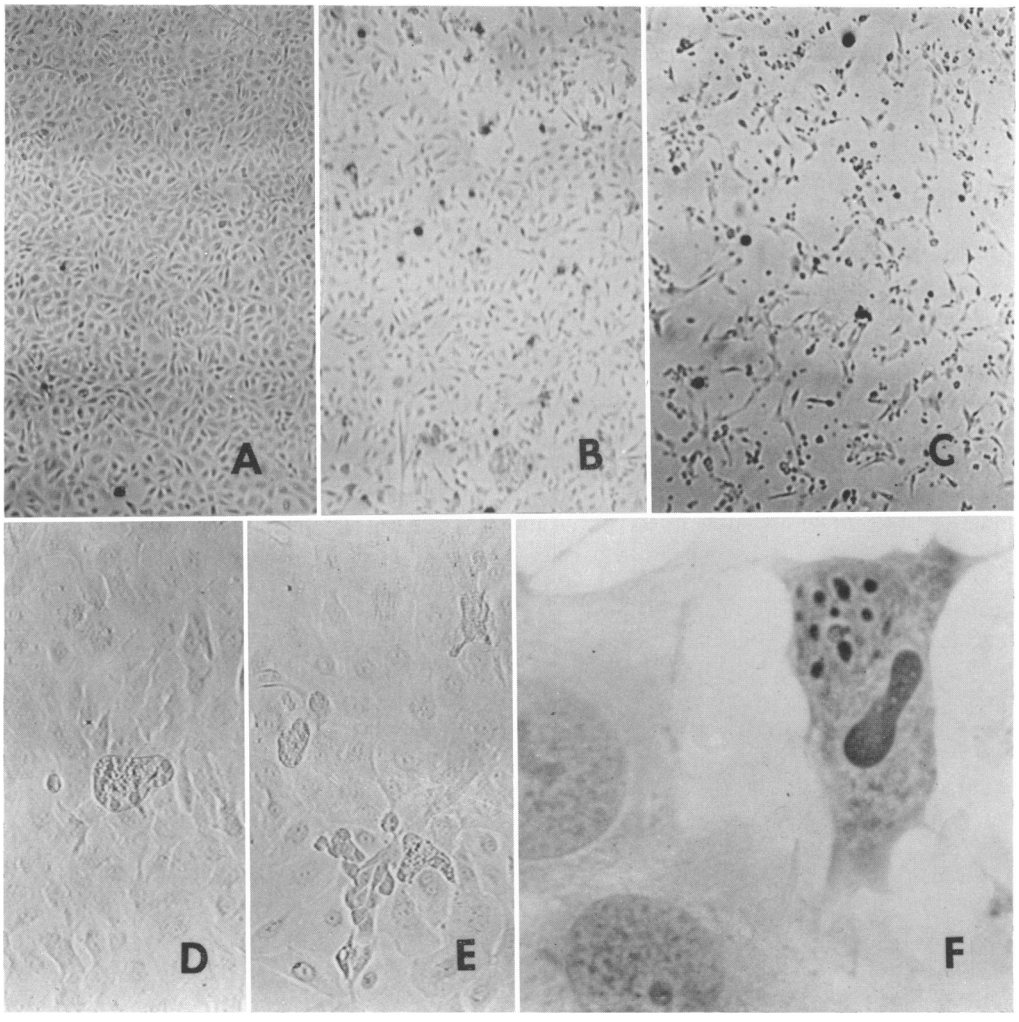

FIG. 1. *Cytopathic effect of rubella virus in human amnion cultures. (A) Appearance of normal amnion cell culture as viewed microscopically under low magnification (33×). (B) Rubella-infected culture with estimated 20% destruction of cells on 14th day after inoculation (55th culture passage, Bell strain; 33×). (C) Rubella infected culture with 80% cell destruction on 28th day after inoculation (53rd culture passage, Bell strain; 33×). (D) Single affected cell with adjacent uninvolved cells on 10th day after inoculation (54th culture passage, Bell strain; 132×). (E) Scattered infected cells showing amoeboid distortion on 10th day after inoculation (54th culture passage, Bell strain; 132×). (F) Infected cell with large eosinophilic cytoplasmic inclusion and basophilic aggregation of nuclear chromatin, as well as portions of two normal cells (4th culture passage, 35th day after inoculation, RW strain; hematoxylin and eosin stain, 3,500×). Reproduced by permission with addenda from F. A. Neva and T. H. Weller* (21).

infectious virus are relatively low, usually in the range of 10^4 to 10^5 ID_{50} per ml. As yet, no complement-fixing, hemagglutinating, or hemadsorbing activity has been demonstrable in rubella-infected cultures. Representative growth curves of rubella virus as obtained by us in human and bovine cell cultures are depicted in Fig. 2.

The exact nature of the interference phenomenon resulting from growth of rubella virus under different in vitro conditions is not yet clear. Parkman et al. reported evidence that in GMK cultures the interference between rubella and ECHO-11 virus is not mediated by interferon (25); others (28) have suggested that interferon

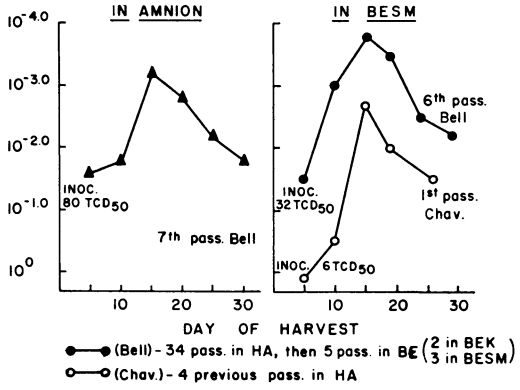

FIG. 2. *Representative growth curves of strains of rubella virus in primary human amnion and in beef embryonic skin-muscle cell cultures.*

does play a role in this system. The interfering activity for Sindbis virus in the fluids of rubella-infected PHA cultures can readily be dissociated from infectious rubella virus, and it also exhibits other properties compatible with interferon (22).

SEROLOGICAL STUDIES WITH RUBELLA VIRUS

Serological procedures for measurement of antibody to German measles virus, and for identification of the agent, are presently limited to neutralization tests. These are applications of the two basic methods for isolation of virus, namely, inhibition of rubella CPE, or inhibition of the rubella interference phenomenon. Technical problems associated with both types of tests are related to factors such as (i) absence of rapid and obvious rubella CPE, (ii) incomplete neutralization of virus by antibody, (iii) critical adjustment of rubella virus dose, and (iv) difficulties in standardization of the interference neutralization procedure. These problems have been discussed in detail elsewhere (22, 26).

Although simplified procedures are needed for routine diagnostic purposes, the methods in current use for assay of neutralizing antibody to rubella have yielded considerable information. Patients with German measles exhibit increases in serum-neutralizing antibody after their illness. The titers of antibody found in individuals who have presumably experienced rubella in the past suggest that substantial levels of antibody are maintained for prolonged periods. The same inference can be drawn from the fact that neutralizing antibody is readily demonstrable by the interference test in specimens of pooled γ-globulin (31, 26, 22).

The first laboratory evidence indicating a considerable degree of antigenic homogeneity of rubella virus strains was the demonstration of antibody responses to recent virus isolates in paired serum samples collected from rubella patients during previous years (36, 21). Similar conclusions regarding the absence of major antigenic differences among rubella strains have been obtained from cross-neutralization tests with antisera prepared in rabbits (25).

CLINICAL AND EPIDEMIOLOGICAL ASPECTS OF RUBELLA

Since the diagnosis of rubella hitherto has been based upon clinical and epidemiological criteria, the advent of laboratory methods for specific diagnosis of rubella infection may be expected to define the clinical spectrum of the disease. There is little, however, at present that can be added to clinical rubella that was not covered in the excellent review of the subject by Wesselhoeft in 1947 (38). Some features of the disease, such as the transitory polyarthritis, have received increased attention recently. The first patient from whom we isolated rubella virus (36) would hardly qualify as a typical example of rubella; he had fever and severe joint symptoms lasting a week, associated with an atypical rash most prominent on the palms of the hands and soles of the feet. Thrombocytopenia accompanying rubella may be deserving of special comment; platelets are often decreased even though bleeding or purpura is not clinically manifest (1, 34), and a number of cases of neonatal purpura following maternal rubella have been reported (3). A point of clinical interest in two adult males with laboratory verified

rubella and thrombocytopenia encountered by us was that the appearance of purpura on the lower legs 3 or 4 days after onset of rash was interpreted as recurrence of exanthem by the patients. The more unusual complications, such as relapses of the disease and rubella encephalitis, can now be investigated in the laboratory; search for virus in the spinal fluid in the latter instance, for example, would be of interest.

In contrast to measles, the clinical diagnosis of rubella is often impossible due to the lack of characteristic signs or symptoms, and variation in extent and nature of the rubella rash. It is understandable that many of the entities on the expanding list of enterovirus exanthems (13) may clinically be labeled as rubella. This was apparent in Massachusetts, during an epidemic of "Boston Exanthem" or ECHO-16 disease in 1951 (20), when a record number of "rubella" cases were notified for the month of August, during an otherwise low-incidence rubella year. Additional evidence of misdiagnosis of rubella can be derived from an analysis of annually and monthly reported rubella in Massachusetts from 1940 to the present time. (We are indebted to Nicholas J. Fiumara, Director of Division of Communicable Diseases, Massachusetts Department of Public Health, for making the monthly and annual tabulations available to us. The recent experience with rubella in Massachusetts is summarized in Fig. 3; by the end of September, the reported number of cases for 1964 was in excess of 36,000.) Customarily, as noted by Ingalls et al. (9), there is a seasonal peak of rubella during the months of March through June in the northern hemisphere. If the monthly distribution of annual cases in Massachusetts during years of high incidence is compared with the plot for years of low rubella incidence, an interesting divergence in patterns results (Fig. 4). A higher proportion of the total cases of "rubella" was reported from August to January during the 8 years of lowest annual incidence than during the corresponding period for the 6 years of highest incidence. Although one cannot exclude the possibility that rubella occurring during the summer and fall may be an inherent epidemiological characteristic of the disease during low periods of endemicity, such a distribution of cases would more likely be expected for years of high rubella incidence. These data incriminate enterovirus exanthems and other viral exanthems as a source of mis-

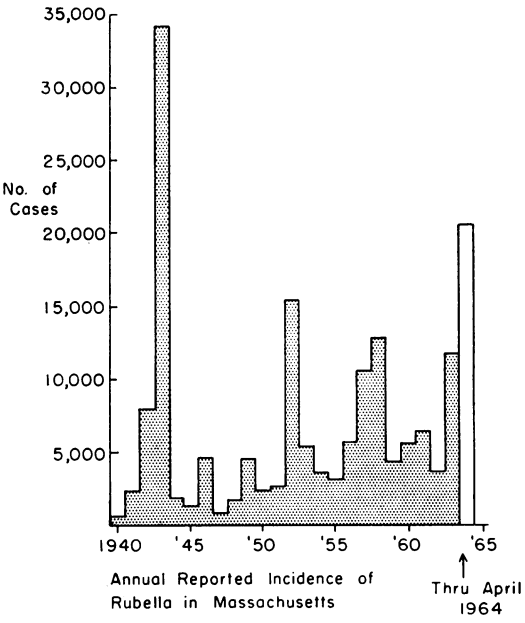

FIG. 3. *Annual reported incidence of rubella in Massachusetts from 1940 to 1964.*

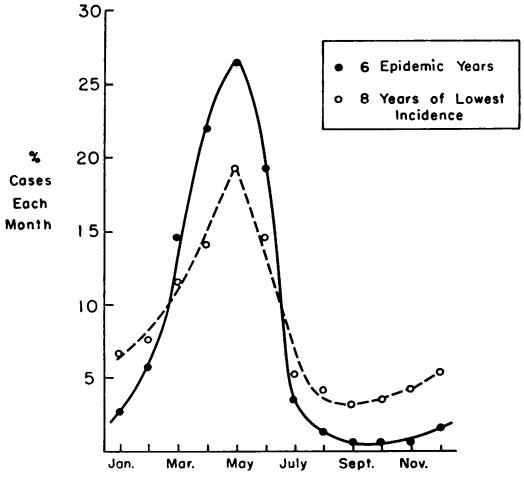

FIG. 4. *Comparison of mean percentage of annual incidence per month of rubella in Massachusetts for 6 epidemic years and for 8 years of lowest incidence.*

diagnosed rubella; they may also help explain Siegel and Greenberg's finding that the risk of congenital rubella was very low during non-epidemic years (32).

Interesting results on the occurrence of subclinical rubella, heretofore an epidemiological hiatus in our understanding of the disease, have

recently come from laboratory-supported studies of rubella in military recruits by Buescher and associates (4). These workers found that 10 to 15% of young adult males from the eastern seaboard entering military service were susceptible to rubella. Then, by carefully following events in two training groups, the ratio of subclinical infection to overt disease was estimated to be 6.5:1. Under these circumstances, virtually all susceptibles became infected. However, the epidemiological pattern of rubella in a civilian setting remains to be determined, for rubella in military recruits may differ from that occurring in civilian populations in a manner somewhat analogous to adenovirus disease. If studies in civilian populations yield results comparable to the situation in military recruits, it would have important implications regarding the problem of maternal rubella.

MATERNAL RUBELLA AND CONGENITAL FETAL DAMAGE

Despite the relative abundance of information concerning the risk of fetal damage following maternal rubella, re-evaluation of the problem is indicated under circumstances whereby the diagnosis of rubella can be verified by laboratory study. Carefully designed and well-controlled prospective investigations (17, 16, and as reviewed in 5) have yielded variable estimates of risk of major congenital defects after maternal rubella during the first trimester of pregnancy. Campbell (5) places the overall risk of abortion and of malformation at 30 to 70% for exposures occurring during the first 4 weeks of pregnancy, declining to 10 to 25% during the fourth 4 weeks of gestation. Such estimates are complicated by the fact that long term follow-up is necessary, as certain defects require the passage of several years before becoming clinically apparent (15, 10). Some experiences of high risk maternal rubella have been attributed to unusually severe epidemics (14, 12). In view of the seasonal pattern and possible misdiagnosis of rubella, referred to earlier, the data from individual epidemics may be more accurate than those from larger, long-term investigations. The possibility that the virulence of rubella virus for the fetus varies from year to year cannot be excluded, but it seems more likely that errors in diagnosis have constituted the major difficulty in accurately assessing the risk of congenital defects subsequent to maternal rubella.

Recent laboratory studies have provided an additional dimension, one with practical applications, to the problem of congenital rubella infection. Two reports (27, 37) indicate that a state of immunological tolerance is not induced in the fetus exposed to rubella virus, and that elevated levels of neutralizing antibody can persist for years in the child with congenital rubella syndrome (37). This finding may be very helpful in the retrospective diagnosis of congenital damage consequent to maternal rubella. Recovery of rubella virus from a human fetus with presumed congenital infection has also been accomplished (29). In our laboratory, rubella virus has been isolated from 24 fetal or placental specimens obtained from 51 women within 49 days after maternal infection. In three instances, furthermore, the virus has been demonstrated postnatally in the pharynx or urine of infants showing stigmata of congenital rubella syndrome. Further definitive investigations of the phenomenon noted by Gregg nearly 25 years ago (6), and which have since directed most of the interest in rubella, may now be expected.

ACKNOWLEDGMENTS

This investigation was supported in part by Public Health Service research grant AI-01023 from the National Institute of Allergy and Infectious Diseases, and by grants from Parke, Davis & Co. and United Cerebral Palsy Research and Educational Foundation, Inc.

LITERATURE CITED

1. ACKROYD, J. F. 1949. Three cases of thrombocytopenic purpura occurring after rubella. Quart. J. Med. N.S. **18**:299–318.
2. ANDERSON, S. G. 1949. Experimental rubella in human volunteers. J. Immunol. **62**:29–40.
3. BERGE, T., F. BRUNNHAGE, AND L. R. NILSSON, 1963. Congenital hypoplastic thrombocytopenia in rubella embryopathy. Acta Pediat. **52**:349–352.
4. BUESCHER, E. L., P. D. PARKMAN, H. L. WEISBERGER, M. S. ARTENSTEIN, F. C. CADIGAN, AND R. E. NITZ. 1964. Studies of rubella. Epidemiology in military recruits. J. Immunol., in press.
5. CAMPBELL, M. 1961. Place of maternal rubella in the aetiology of congenital heart disease. Brit. Med. J. **1**:691–696.

6. GREGG, N. M. 1941. Congenital cataract following German measles in the mother. Trans. Ophthalmol. Soc. Australia (BMA) **3**:35–46.
7. HABEL, K. 1942. Transmission of rubella to *Macacus mulatta* monkeys. Public Health Rept. **57**:1126–1139.
8. HIRO, Y., AND S. TASAKA. 1938. Die Röteln sind eine Viruskrankheit. Monatsschr. Kinderheilk. **76**:328–332.
9. INGALLS, T. H., F. L. BABBOTT, JR., K. W. HAMPSON, AND J. E. GORDON. 1960. Rubella: its epidemiology and tetralogy. Am. J. Med. Sci. **239**:363–383.
10. JACKSON, A. D. M., AND L. FISCH. 1958. Deafness following maternal rubella. Results of a prospective investigation. Lancet **2**: 1241–1244.
11. KRUGMAN, S., R. WARD, K. G. JACOBS, AND M. LAZAR. 1953. Studies on rubella immunization. 1. Demonstration of rubella without rash. J. Am. Med. Assoc. **151**:285–288.
12. LAMY, M., AND M. E. SEROR. 1956. Resultats d'une enquête sur les embryopathies d'origine rubéolique. Bull. Acad. Natl. Med. (Paris). **140**:196–203.
13. LERNER, A. M., J. O. KLEIN, J. D. CHERRY, AND M. FINLAND. 1963. Medical progress. New viral exanthems. New Engl. J. Med. **269**:678–685, 736–740.
14. LIGGINS, G. C., AND L. I. PHILLIPS. 1963. Rubella embryopathy. An interim report on a New Zealand epidemic. Brit. Med. J. **1**:711–713.
15. LOCK, F. R., H. B. GATLING, AND H. B. WELLS. 1961. Difficulties in the diagnosis of congenital abnormalities. Experience in a study of the effect of rubella in pregnancy. J. Am. Med. Assoc. **178**:711–714.
16. LUNDSTRÖM, R. 1962. Rubella during pregnancy. A follow-up study of children born after an epidemic of rubella in Sweden, 1951, with additional investigations on prophylaxis and treatment of maternal rubella. Acta Pediat. Suppl. **133**:1–110.
17. MANSON, M. M., W. P. D. LOGAN, AND R. M. LOY. 1960. Rubella and other virus infections during pregnancy. Reports on Public Health and Medical Subjects. No. 101, Ministry of Health, London, Her Majesty's Stationery Office. 1–101.
18. MATON, W. G. 1815. Some account of a rash, liable to be mistaken for scarlatina. Med. Trans. Roy. Coll. Phys. **5**:149–165.
19. MCCARTHY, K., C. H. TAYLOR-ROBINSON, AND S. E. PILLINGER. 1963. Isolation of rubella virus from cases in Britain. Lancet **2**:593–598.
20. NEVA, F. A., R. F. FEEMSTER, AND I. J. GORBACH. 1954. Clinical and epidemiological features of an unusual epidemic exanthem. J. Am. Med. Assoc. **155**:544–548.
21. NEVA, F. A., AND T. H. WELLER. 1964. Advances in our knowledge of rubella, p. 90–95. *In* Industry and tropical health. Industrial Council for Tropical Health, Harvard School of Public Health, Boston.
22. NEVA, F. A., AND T. H. WELLER. 1964. Rubella interferon and factors influencing the indirect neutralization test for rubella antibody. J. Immunol., in press.
23. NORRBY, E., R. MAGNUSSON, B. FRIDING, AND S. GARD. A note on the morphology of rubella virus. Arch. Ges. Virusforsch. **13**:421–424.
24. PARKMAN, P. D., E. L. BUESCHER, AND M. S. ARTENSTEIN. 1962. Recovery of rubella virus from army recruits. Proc. Soc. Exptl. Biol. Med. **111**:225–230.
25. PARKMAN, P. D., E. L. BUESCHER, M. S. ARTENSTEIN, J. M. MCCOWN, AND F. K. MUNDON. 1964. Studies of rubella. I. Properties of the virus. J. Immunol., in press.
26. PARKMAN, P. D., J. M. MCCOWN, F. K. MURDON, A. DRUZD, AND E. L. BUESCHER. 1964. Studies of rubella. II. Neutralization of the virus by immune sera. J. Immunol., in press.
27. PLOTKIN, S. A., J. A. DUDGEON, AND A. M. RAMSAY. 1963. Laboratory studies on rubella and the rubella syndrome. Brit. Med. J. **2**:1296–1299.
28. ROZEE, K. R., K. F. GIVAN, F. W. DOANE, AND A. J. RHODES. 1963. A plaque method for rubella virus assay. Can. Med. Assoc. J. **89**:314–315.
29. SELZER, G 1963. Virus isolation, inclusion-bodies, and chromosomes in a rubella infected human embryo. Lancet **2**:336–337.
30. SEVER, J. L., G. M. SCHIFF, AND R. G. TRAUB. 1962. Rubella virus. J. Am. Med. Assoc. **182**:663–671.
31. SCHIFF, G. M., J. L. SEVER, AND R. J. HUEBNER. 1963. Rubella virus: neutralizing antibody in commercial gamma globulin. Science **142**:58–60.
32. SIEGEL, M., AND M. GREENBERG. 1960. Fetal death, malformation, and prematurity after maternal rubella. New Engl. J. Med. **262**: 389–393.
33. SIGURDARDOTTIR, B., K. F. GIVAN, K. R. ROZEE, AND A. J. RHODES. 1963. Association of virus with cases of rubella studied in

Toronto; propagation of the agent and transmission to monkeys. Can Med. Assoc. J. **88**:128–132.

34. STEEN, E., AND K. H. TORP. 1956. Encephalitis and thrombocytopenic purpura after rubella. Arch. Disease Childhood **31**:470–473.
35. VERONELLI, J. A., H. F. MAASSAB, AND A. V. HENNESSY. Isolation in tissue culture of an interfering agent from patients with rubella. Proc. Soc. Exptl. Biol. Med. **111**:472–476.
36. WELLER, T. H., AND F. A. NEVA. 1962. Propagation in tissue culture of cytopathic agents from patients with rubella-like illness. Proc. Soc. Exptl. Biol. Med. **111**:215–225.
37. WELLER, T. H., C. A. ALFORD, JR., AND F. A. NEVA. 1964. Retrospective diagnosis by serologic means of congenitally acquired rubella infection. New Engl. J. Med. **270**: 1039–1041.
38. WESSELHOEFT, C. 1947. Rubella (German measles). New Engl. J. Med. **236**:943–950, 978–988.